EXHIBIT F

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Illana Gozes et al.

Application No.: 10/748,765

Filed: December 29, 2003

For: METHODS OF TREATING

AND/OR PREVENTING AUTOIMMUNE DISEASES

Customer No.: 20350

Mail Stop Amendment Commissioner for Patents P.O. Box 1450 Alexandria, VA 22313-1450 Confirmation No. 8714

Examiner:

C. M. Woodward

Technology Center/Art Unit: 1647

DECLARATION OF DR. ILLANA GOZES

UNDER 37 C.F.R. §1.132

Sir:

I, Illana Gozes, Ph.D., being duly warned that willful false statements and the like are punishable by fine or imprisonment or both (18 U.S.C. § 1001), and may jeopardize the validity of the patent application or any patent issuing thereon, state and declare as follows:

- 1. All statements herein made of my own knowledge are true, and statements made on information or belief are believed to be true and correct.
- I am Director of the Adams Super Center for Brain Studies & Edersheim Levi-Gitter fMRI Institute, also at Tel Aviv University. I have been the incumbent of the Lily and Avraham Gildor Chair for the Investigation of Growth Factors at Tel Aviv University, since 1997. I am also the Chief Scientific Office and Director at Allon Therapeutics, Inc. in Vancouver, Canada. I received a Ph.D. from The Weizmann Institute of Science in 1979, and was a Haim Weizmann Postdoctoral Fellow at the Massachusetts Institute of Technology from 1979-1980. I was a Research Associate and Visiting Scientist at the Salk Institute and the Scripps Clinic and

Research Foundation from 1981-1982. I was a Senior Scientist/Associate Professor at The Weizmann Institute of Science from 1982-1989. I was a visiting scientist in developmental neurobiology at NICHD, NIH from 1989-1990. My affiliation with Tel Aviv University began in 1990. I was a Fogarty-Scholar-in-Residence at NIH from 1995-1996 and an adjunct scientist in developmental neurobiology at NIH from 2003-2004.

- Juludan Prize and the Teva Founders Prize for exceptional scientific studies and the Bergmann Prize and the Neufeld award for outstanding/leading US-Israel BSF grant proposals. I am currently Editor-in-Chief of The Journal of Molecular Neuroscience and I currently sit on the editorial boards of the American Journal of Alzheimer's Disease, the International Journal of Peptide Research & Therapy and the journal Peptides. I am an author on more than 197 research papers and am an author or co-author of numerous reviews and book chapters. A copy of my curriculum vitae is attached hereto as Exhibit G and includes a list of selected publications.
- 4. The present invention is a method of treating multiple sclerosis (MS) by administering a therapeutically effective amount of an ADNF III peptide to a patient. The treatment includes administration of a peptide that comprises the core active site sequence of ADNF III, *i.e.*, the amino acid sequence NAPVSIPQ known as "NAP." NAP is the smallest peptide that exhibits the same activity as full-length ADNF III. Use of D-amino acid NAP and ADNF III peptides for treatment of MS are also claimed.
- 5. I have read and am familiar with the contents of this patent application. In addition, I have read an Office Action, dated April 29, 2008, received in the present case, as well as the cited references. It is my understanding that the Examiner alleges that the claimed invention is obvious in view of Gozes *et al.*, US patent No. 6, 613,740, WO98/35042 and Brenneman *et al.*, US 2002/0111301; or Brenneman *et al.*, Gozes *et al.*, US Patent 4,587,046 and Voet *et al.*, Biochemistry 2nd Ed., page 67. Specifically, the Examiner states that one of skill would have predicted that ADNF III polypeptides could be used to treat MS because the cited references disclose the ADNF III polypeptides can be used to treat the neuro-auto immune disorder Guillian-Barre syndrome. The Examiner also states that one of skill would have a

reasonable expectation of success because ADNF peptides treat conditions related to neuronal cell death.

- 6. This declaration is provided to demonstrate that the cited references do not provide motivation for their combination to arrive at the claimed methods and do not predict that the claimed methods would result from their combination. Moreover, the invention is based on a surprising result: ADNF III peptides inhibit the proliferation of immune cell and decrease the levels of cytokines secreted by immune cells.
- 7. Before the filing date of the priority application, ADNF III was known to prevent neuronal cell death. The ability of ADNF III to inhibit neuronal cell death was first demonstrated in vitro using isolated neuronal cells. *See, e.g.*, US Patent No. 6,613,740, Gozes *et al.*, Figs. 6A-C, 7A-B and column 58, line 23 through column 59, line 4. Thus, other cell types, *e.g.*, immune cells, are not required for inhibition of neuronal cell death by ADNF III.
- 8. Before the publication of the present application, it was not known that ADNF III affects non-neuronal cells, including immune cells. It was not known that ADNF III inhibits of proliferation of immune cells. It was not known that ADNF III decreases the amount of cytokines, e.g., of tumor necrosis factor- α (TNF- α) and interleukin-12 (IL-12) secreted by immune cells. These new activities of ADNF III are disclosed in the specification and are also confirmed in post-filing data.
- 9. The specification provides evidence that ADNF III peptides inhibit proliferation of immune cells using an art accepted animal model of MS, myelin-oligodedrocyte glycoprotein (MOG)-induced chronic experimental autoimmune encephalomyelitis (EAE) in mice.
- 10. The specification demonstrates the effect of ADNF III peptides on the MOG-induced chronic EAE model at paragraphs [103]-[106]. EAE was induced by immunization of mice with the peptide encompassing amino acids 35-55 of rat MOG. Peptide synthesis was carried out by the Weizmann Institute Synthesis Unit using a solid-phase

technique, on a peptide synthesizer (Applied Biosystems Inc., Foster City, CA City). Six weeks old C57/b mice (Tel-Aviv University) were injected (subcutaneous) in the flank with a 200 μ l emulsion containing 300 μ g MOG peptide in complete Freund adjuvant (CFA) and 500 μ g Mycobacterium tuberculosis (Sigma Israel). An identical booster immunization was given on the other flank one week later. Ten days following the encephalitogenic challenge, the MOG-treated mice were observed daily and the clinical manifestations of EAE were measured by the following score: 0 = 10 clinical symptoms; 1 = 10 so of tail tonicity; 1 = 10 partial hind limb paralysis; 1 = 10 paralysis; 1

- microgram/mouse in a mixture containing 7.5 mg/ml sodium chloride, 1.7 mg/ml citric acid monohydrate, 3.0 mg/ml disodium phosphate dehydrate and 0.2 mg/ml of a 50% benzalkonium chloride solution. The nasal administration was given daily, 1 hour after MOG injection and was continued and given once a day, 1 hour prior to testing. Control animals received the above mixture without NAP. In the example here, NAP's daily treatment began 10-14 days prior to the MOG injection. Results showed that NAP significantly improved the clinical outcome of the animals, day 11 on, P<0.01, t-test (Figure 1 of the specification).
- Results indicated that NAP inhibited the immune response (cell proliferation, Fig. 2 of the specification) *in vivo* as the proliferative response of splenocytes was much reduced (P<0.01) in the mice treated with NAP as compared to untreated mice. Furthermore, addition of MOG resulted in increased proliferation in the splenocytes of untreated animals, even at 2 micrograms/well of MOG, P<0.05). In contrast, even at 25 micrograms MOG, the proliferative response NAP treated animals did not increase.
- 13. Post-filing results confirm the ability of ADNF III peptides to decrease levels of tumor necrosis factor- α (TNF- α) and interleukin-12 (IL-12) and also confirm the anti-proliferative effect of ADNF III peptides on T-cells that are activated by a MOG antigen.

- 14. Quintana *et al.*, *Ann. N.Y. Acad. Sci.* 1070:500-506 (2006) is submitted as Exhibit H. RAW 264.7 cells, a transformed macrophage cell line, were activated by LPS in the presence or absence of an ADNF III peptide. Results are shown at page 503 and Figure 2. As levels of ADNF III peptide were increased, levels of TNF-α and IL-12 secreted by the RAW 264.7 cells decreased. Thus, Quintana *et al.* demonstrate that ADNF III peptides decrease levels of TNF-α and IL-12 secreted by immune cells. As these experiments were done using isolated immune cells, the demonstrate that the ADNF III effect on immune cells is independent of its effect on neuronal cell.
- 15. The references cited by the Office Action demonstrate that ADNF III peptides prevent neuronal cell death and are silent on an ADNF III function related to immune cells. In my opinion, the ability of ADNF III to prevent neuronal cell death did not suggest or predict that ADNF III would inhibit cell proliferation, including immune cell proliferation. Prevention of neuronal cell death by ADNF III did not suggest or predict that administration of ADNF III would independently decrease the levels of cytokines secreted by immune cells. Therefore, in my opinion, one of skill would consider the discovery that ADNF III peptide inhibits immune cell proliferation to be a surprising result.

	16.	In view of the forgoing, in my opinion, the cited references do not teach,
suggest, or pro	edict the	e claimed method to treat MS. In addition, the effect of ADNF III peptides
on the immun	e respor	nse was a surprising result.

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Date: _	September 25, 2008	By:		U	

Illana Gozes, Ph.D.

BLK:blk 61473267 v161473267 v1

EXHIBIT G

Name, Titles & Address

Illana Gozes, Ph.D.

Professor of Clinical Biochemistry
The Lily and Avraham Gildor Chair
for the Investigation of Growth Factors

Director of the Adams Super Canter for Brain Studies

& the Levie-Edersheim Gitter Institute for Functional Brain Imaging

Head, Section of Clinical Biochemistry & the Dr. Diana and Zelman Elton (Elbaum) Laboratory for Molecular Neuroendocrinology

Department of Human Molecular Genetics and Biochemistry

Sackler School of Medicine

Tel Aviv University Tel Aviv, Israel Tel: 972-3-640-7240 Fax: 972-3-640-8541

e-mail: igozes@post.tau.ac.il

Consultant:

Chief Scientific Officer, Allon Therapeutics Inc. Editor in Chief, Journal of Molecular Neuroscience

Education:

1969-1972 Tel Aviv University Biology, Life Sciences B.Sc., 1973 1973-1978 Feinberg Graduate School, Neurobiology Ph.D., 1979

of the Weizmann Institute of Science.

Ph.D. Thesis: The control of tubulin expression in the developing nervous system.

Supervisor: Prof. U.Z. Littauer.

ACADEMIC AND PROFESSIONAL EXPERIENCE:

Period:	Name of Institution:	Department:	Rank/Function:
1979-1980	Massachusetts Institute	Laboratory of	Postdoctoral
	of Technology (MIT)	Neuroendocrine	Fellow
	Cambridge, MA, USA.	Regulation.	
1981-1982	The Salk Institute,	Behavioral	Research
	and The Scripps Clinic	Neurobiology	Associate,
	and Research Foundation.		Visiting
	San Diego, CA, USA		Scientist.
1982-1987	The Weizmann Institute	Hormone	Senior
	of Science.	Research	Scientist.
1987-1989	The Weizmann Institute	Hormone	Associate
	of Science.	Research	Professor.
1988-1989	NICHD, NIH.	Lab. Molecular	Sabbatical.
	Bethesda, MD, USA	Genetics.	
1989-	NICHD,NIH	Developmental	Visiting
	Bethesda, MD, USA	Neurobiology	Scientist.
		Invited Gues	t Scientist (Summers)
1990-	Tel Aviv University	Chemical Path.	Associate
	Tel Aviv, Israel	Dept.	Professor
1993-94	Tel Aviv University	Chemical Path.	Chairperson
	Tel Aviv, Israel	Dept.	O
1993-4	Tel Aviv University	Sackler Faculty	Chairperson
	Tel Aviv, Israel	of Medicine,	Preclinical

			Department
			Head
1993-	Tel Aviv University		Professor
	Tel Aviv, Israel		of Clinical
	,		Biochem.
1994-5	Tel Aviv University	Clinical Biochem.	Chairperson
	Tel Aviv, Israel	Dept. (formerly Chem. Path.)	
1995-6	NIH	Fogarty International Center	
1997-	Tel Aviv University	3	The Lily and Avraham Gildor
	Tel Aviv, Israel		Chair for the Investigation of
			Growth Factors
1998-2002	Tel Aviv University	Sackler Faculty	Head, International Advisory
1000 2002	Tel Aviv, Israel	of Medicine,	Committee
	10171111, 101401	J	Member, Committee of
			School heads (-2000)
2001-2003	Allon Therapeutics, Inc.		Chief Executive Scientist
2001-2000	San Diego, CA, USA		Chair of the Board
2003-2004	Allon Therapeutics, Inc.		Chief Scientific Officer
2003-2004	San Diego, CA, USA		Vice Chair of the Board
	Sall Diego, CA, COA		Chair, Scientific Adv. Board
			interim CEO (summer 2004)
2004-	Allon Therapeutics, Inc.		Chief Scientific Officer
2004-	Vancouver, BC, Canada		Director; Chair, Scientific
	varicouver, BC, Carrada		Adv. Board (consultant)
0000 0004	NICHD NIII Betheede MD	LICA NICHD	Adjunct Scientist
2003-2004	NICHD, NIH, Bethesda, MD,	Sackler Faculty	The Dr. Diana and Zelman
2004-	Tel Aviv University	of Medicine	Elton (Elbaum) Laboratory
	Tel Aviv, Israel	of Medicine	Molecular Neuroendocrin.
2000	Tol Aviv University		Director,
2006 -	Tel Aviv University		The Adams Super Center for
•	Tel Aviv, Israel		Brain Studies
			The Levie- Edersheim-Gitter
			Institute, for Functional Brain
			Imaging

2007

Israel Society for Neuroscience

President Elect

Teaching at the Feinberg Graduate School, The Weizmann Institute of Science

1977	Laboratory course on Protein Synthesis,	Instructor.
1978	Laboratory course on Gel Electrophoresis,	Instructor.
1982	Laboratory course on Basic Methods in Biological Resear	ch, Course Organizer and Instructor.
1984	Lecture course on Molecular Biology of Neuropeptides.	
1986	Seminar course (joined) on Neuropeptides.	

Teaching at Sackler School of Medicine, Tel Aviv University

1991- Seminar course (joined) on: Neuropeptides: from molecular genetics to function (biennial).

Cellular peptides and proteins lectures in the course on Chemical Pathology.

1993- Selected lectures in Chemical Pathology

Selected lectures in Biochemistry

Appointed:

2007-

Course coordinator in:

Biochemistry for the New York Program of the Sackler School of Medicine.

Chemical Pathology for the Israeli program.

1995-7 Appointed: course coordinator in: Biochemistry

1998- Intercellular communicators: neuropeptides and growth hormones (biennial).

1999- Models for neurodegenerative diseases (biennial).

2003- Course coordinator in: The molecular basis of diseases.

Administrative and Academic Responsibilities (selected)

1992-1995	Sackler Faculty officer in-charge of the radioactivity safety.
1993-1995	Sackler Faculty of Medicine, Department Head.
1997-2001	Tel Aviv University Senate Committee for master degrees
1998-	Sackler Faculty officer, in charge of international interactions: member, heads of Schools committee and the School of Medicine governing committee.
1999-	Sackler Faculty pre-clinical appointments and promotion committee.
2000-	Co-Chair, The American Summer Neuropeptide Confrence
2001-	Co-Director (with Dr. J.P. Schwartz): NIH-Tel Aviv University Graduate Program in Women Health Studies).
2002-	Tel Aviv University Senate Committee for postdoctoral fellows Sackler Faculty of Medicine, Tel Aviv University, Grants Committee
2003-	Secretary General, the European Neuropeptide Club (ENC).
2005-	Ad Hoc Committee for application of patent laws (Tel Aviv University)
2006-	Member, Tel Aviv University Board of Governors; Professorial Chairs Committee, Sackler Faculty of Medicine, University Committee for Women Issues.

For additional administrative and academic responsibilies, please see below in – membership and leadership in professional societies.

Member, Search committee for the dean of Life Sciences

Head, Unit of Clinical Biochemistry, Sackler Faculty of Medicine, Tel Aviv University

D. ACTIVE PARTICIPATION IN SCIENTIFIC MEETINGS:

1979 Cytoskeleton Meeting, Cold Spring Harbor, USA. Strasburg-Weizmann Meeting, Rehovot, Israel. 1983 Israel-France Neuroendocrine Workshop, Rehovot, Israel. Regional Meeting of the International Union of Physiological Science, Jerusalem, 1984 Israel. Neuroendocrinology Symposium, Rehovot, Israel. IBRO/UNESCO Workshop on: Mechanism of Secretion and action of 1985 Neurotransmitters & Neuromodulators in Central and Peripheral Synapses, Jerusalem, Israel. Neuropsychopharmacology Group Symposium on Neuropeptides and Neuropeptide Genes. Albert Einstein College of Medicine, NY, USA. Neuroscience Colloquium, Israel ETP Autumn School. Brain Imaging: New Approaches to the Study of Brain Function. 1986 Greece. Neuropeptide Symposium - Israel Endocrine Society Meeting, Israel. Vasoactive Intestinal Peptide and Related Peptides. A New York Academy Sponsored 1987 Meeting. NY, USA. 11th Pasteur-Weizmann Symposium onRecent Developments in Neurobiology, Rehovot, Israel. ETP/ENA/IBRO Practical Course on Molecular Neuroanatoanatomical Techniques, Amsterdam, Holland. From Gene to Behavior: New Approaches to the Study of Brain Function, ETP Course, Italy. Molecular Approach to Hormone Action. Teberias, Israel (Organizing Committee). European Society for Neurochemistry, Sweden (also - Invited Symposium 1988 Chairperson). International Society for Developmental Neuroscience Jerusalem, Israel (Also Invited Chairperson). The second Otto Loewi Meeting on Cellular and Molecular Neurobiology, Eilat, Israel. 1989 The 4th International Symposium on VIP and Related Peptides. Sweden. R.M. Goodman International Conference 1990

New Perspectives on Genetic Markers and Genetic Diseases Among the Jewish People. Neve Ilan, Israel.

Environmental Determinants of Nervous System Development, Birmingham, Alabama, USA (also - Invited Chairperson).

1991

Annual meeting of the Israeli Societies for Clinical Chemistry, Biochemistry and Endocrinology, Tel Aviv, Israel.

Recent Advances in Neuroendocrine Regulatory Mechanisms. Israel Academy of Science and Humanities. Jerusalem, Israel.

Genetic Basis for Neuropsychiatric Disorders. Tel Aviv, Israel.

1992

Growth Factors, Peptides and Receptors'92, The 12th Washington International Spring Symposium. Washington, D.C., USA.

International Society for Developmental Neuroscience, Montpelier, France.

Israel Society for Clinical Biochemistry, Haifa, Israel.

1993

Winter Neuropeptide Symposium, CO, USA.

European Neuroendocrinology Meeting, Lisbon, Portugal.

Israeli Society for Clinical Biochemistry, Israel.

Israel-Poland Science meeting (Poland)

VIP,PACAP & Related Regulatory Peptides From Molecular Biology to Clinical Application. Strasbourg, France.

Israeli Society for Clinical Biochemistry: Organizer of a short meeting on Cell Death and Proteins Associated with Life and Death Decisions Tel Aviv, Israel.

NICHD: Genetics and Behavior Workshop. Bethesda, MD, USA.

The Second INSERM-WEIZMANN Conference on Molecular Endocrinology, from Geneto the Clinic. Rehovot, Israel.

1994

Winter Neuropeptide Symposium, CO, USA.

Organizer, Growth Factors, Hormones and New Drugs. Supported by the Israeli Ministry for Research and Technology. (D.E. Brenneman, International Associate Symposium Chairperson).

Summer Neuropeptide Conference, Invited Chairperson, MA, USA.

Israeli Society for Endocrinology: Molecular Endocrinology Meeting, Invited Organizer.

Israeli Society for Gastroenterology (invited speaker). Israel

European Neurochemistry Society (ESN) - invited session organizer. Jerusalem, Israel.

Society for Neuroscience, Invited symposium speaker. Miami Beach, Florida.

1995

Chairperson of the Scientific Organizing Committee, 7th Meeting of the European Neuroendocrine Association and the Adams Workshop on Gene Transfer in the Brain.

Israeli Society for Clinical Biochemistry: Annual meeting, invited symposium chairperson. Israel.

Israel-Poland Science Meeting, Israel.

Center for Cancer Research, Tel Aviv University, Eilat, Israel.

VIP, PACAP & Related Regulatory Peptides From Molecular Biology to Clinical Application. New Orleans, LA, USA.

International Society for Neurochemistry

15th Biennial Meeting (SymposiumChairperson), Kyoto, Japan.

VIP/PACAP family of neuropeptide receptors, Osaka, Japan.

1996

Winter Neuropeptide Symposium, CO, USA.

Summer Neuropeptide Conference, MA, USA.

National Polish Menopause and Adropause Soc. Poland (Plenary speaker).

1997

Neuropeptides in Sensory and Other brain Systems, Kitzbuhel, Austria (Symposium Chair).

Stress of Life-International Congress of Stress, Budapest, Hungary (Symposium Co-Chair).

Alzheimr's Disease meetings (Israel).

The VIP/PACAP and related peptides (Freiburg, Germany)

Organizing Committee: Signal Transduction in Health and Disease (Tel Aviv, Israel)

1998

Winter Neuropeptides (Symposia Organizer, Colorado, USA)

Minerva Meeting (Neuroscience, Israel)

Summer Neuropeptides (Gent, Belgium)

European Society for Neurochemistry (Symposia Organizer, St. Petersburg, Russia)

18th Minerva Symposium, New Strategies and Perspectives in understanding CNS disorders (Jerusalem, Israel).

Third Sepharadi-Israel meeting (Salamanca, Spain)

Organizing Committee: The 5th IUBMB Conference on The Biochemistry of Health and Disease (Jerusalem, Israel).

International Society for Neuroendocrinology meeting (Japan).

International Workshop on Conformational Diseases (Israel).

The Israeli Society for Neurosciences (Organizing Committee, Eilat, Israel).

1999 Winter Neuropeptides (Colorado, USA)

American Society for Neurochemistry (New Orleans, LA, USA)

Pathophysiology of the Brain and the nervous System (The second

Doris Cecelia Levy Memorial Seminar, Kiryat Anavim, Israel).

Neuropeptides-Ferrara 1999 (Italy)

Scietific advisory board: International Symposium on VIP and PACAP and Related

Peptides (Elsinore, Denmark)

The Israeli Society for Neurosciences (Organizing Committee, Eilat, Israel).

Sackler Faculty of Medicine, Research Fair (Tel Aviv, Israel)

Winter Neuropeptides (Symposium organizer; Colorado, USA)

European Neuropeptide Club (Symposium organizer; Innsbruck, Austria)

Summer Neuropeptide Conference, Sainte-Adele, Quebec, Canada

Organizer: The second Saul Farber Symposium, the New York State/ American Program of the Sackler Faculty of Medicine.

The 7th Tel Aviv University Alzheimer Conference, Tel Aviv University / Tel Aviv Sourasky Medical Center.

Organizing Committee: Signal Transduction in Health and Disease

The Blood-Brain Barrier: Oholo Conference (Dead Sea, Israel)

The Gentner Symposium on Cellular And Molecular Processes In Biological Signaling (Jerusalem, Israel)

Investigators Meeting, Institute for the Study of the Aging (New York, USA)

Cytokines Meeting (New Orleans, USA)

Organizer: Neuropeptides 2001: the European Neuropeptide Club and the American Summer Neuropeptide Meeting (Maale Hachmisha, Israel)

Winter Neuropeptides (Symposium organizer; Colorado, USA),

Israel-Germany meeting on Signal Transduction (Berlin, Germany)

The 8th Tel Aviv University Alzheimer Conference, Tel Aviv University / Tel Aviv Sourasky Medical Center

Peptide Receptors (Montreal, Canada).

International Congress of the Polish pharmacological Society (Plenary lecture, Cracow, Poland)

Investigators Meeting, Institute for the Study of the Aging (New York, USA)

VIP, PACAP and related peptides meeting (Platform lecture, Santa Barbara, CA, USA);

ISOA investigators meeting (NY, USA).

Winter Neuropeptides (Symposium organizer; Colorado, USA)

2000

2001

2002

European Neuropeptide Club (Symposium organizer, Scientific Advisory Committee, Poland).

American Summer Neuropeptides (Co-Chair, Florida, USA)

ECNP (European College of Neurophychopharmacology) Congress (Barcelona, Spain)

STUDYIII (Israel, organizing committee); Canada-Israel Neuroscience, organizing session); ISOA investigators meeting (NJ, USA).

2003 VIP, PACAP and related peptides meeting (Scientific Advisory Committee, Japan);
American Summer Neuropeptides (Co-Chair, NY, USA); Winter Neuropeptides
(Colorado, USA), ISOA investigators meeting (NJ, USA), Vascular Dementia (Prague,

Czehc Reublic).

American Summer Neuropeptides (Co-Chair, NY, USA); Winter Neuropeptides (Organizing Committee, Symposium Chair, Colorado, USA) Int'l Biological Psychiatry (Sydney, Australia); Nasal Delivery (London, UK); ENC (Alicante, Spain); ISOA neuroprotection NY Acad. Sci. (NY, USA); International Endocrinology (lisbon. Protugal), International Peptide (Prague, Czehc Reublic), ISOA investigators meeting

(NJ, USA)

2005 American Summer Neuropeptides (Co-Chair, NY, USA); Winter Neuropeptides (Organizing Committee, Symposium Chair, CO, USA)Nasal delivery (DC, USA); Int'l VIP, PACAP (Rouen, France); ISOA Alzheimer (NY, USA); STADY (Tel Aviv, Israel);

SRI:Peptides Therapeutics (Advisor; Philadelphia, USA).

Winter Neuropeptides (Symposium Chair, + two invited lectures, CO, USA), CNS Drug Discovery, Boston, MA, USA; Summer Neuropeptide Conference and ENC (Maimi

Florida, Meeting Chair), ICAD (International Conference on Alzheimer's Disease), Madrid, Spain, Gordon Research Conference of Protein Processing, Israel Society for

Neuroscience (ISFN) Eilat, Israel

2007 Drug Discovery, Development & Delivery for Chronic Neurodegenerative Disease: A

Course for Academic and Biotechnology Scientists. New York, NY, Presentation, Advisory Board; Heart and Brain – Tel Aviv University, AD/PD 2007, Salzburg, Austria; European Neuropeptide Club, Annual Meeting, Santorini Island, Greece, Secretary General, VIP-PACAP Meeting, Vermont, USA, Presentation/Session-Chair/Organizing Committee, Summer Neuropeptide Meeting, Presentation/Meeting Co-Chair (President), Israel Society for Neuroscience, Austrian Society for Alzheimer's

Disease

2008 ENC joint meeting, Ferrara, Italy, The 2nd Joint US-Israeli- Palestinian Brain Conference

Jerusalem, ICAD Chicago, IL, USA; Drug Discovery & Development of Innovative Therapeutics, Boston, MA, USA and multiple lectures in universities in Israel and

worldwide

Active participation in other numerous meetings (e.g. Annual Neuroscience meetings, USA) along the years, with either a short lecture or a poster presentation), + invited lectures in Harvard, Yale, MIT, Caltech, Scripps Clinic, Salk Institute, Stanford, UCSF, UCSB, NIH, Michigan Univ., NYU, Case Western, John Hopkin's, USU, Rutgers, Kings College (London), Scotland University, Singapore National University, China, Beijing Medical University, Germany, University of Regensburg, Japan, Fujimoto Pharmaceutical Corp., Pharmaceutical Corp.: Cephalon, Regeneron, Pfizer, Bayer, Novartis, Johnson & Johnson, Amgen, QLT.

E. ACADEMIC AND PROFESSIONAL AWARDS

1977	Landau Prize (Miphal Ha-pais) For Tel Aviv, Israel. Excellent Ph.D. Thesis.
1977	Awarded a Katzir Fellowship. Rehovot, Israel.
1978-1980	Awarded the Chaim Weizmann Fellowship, Rehovot, Israel.
1978	European Molecular Biology Organization, Heidelberg, Germany.
1982-1984	Jewish Agency Award. Jerusalem, Israel.
1982-1985	The Bergmann Memorial Fund Research Prize. For excellent US-Israel Binational Science Foundation Grant Application.
1983-1989	The first incumbent of Samuel O. Freedman Career Development Chair established by the Montreal Research Chapter of Friends of the Weizmann Institute of Science.
1991	The Juludan Prize for outstanding research achievement which show promise of having valuable scientific-technological application and are channeled to enhance man's welfare and prolong the human life span.
1992	The Israeli Society for Clinical Biochemistry, Best poster award.
1993	The Israeli Society for Clinical Biochemistry, best posters awards (shared with R. Glazer and Dr. G. Lilling).
	Teva Founders Prize for opening new horizons in medical research in Israel (shared with Prof. M. Fridkin).
1995	Fogarty International Scholar
1997	The Israeli Society for Clinical Biochemistry, best posters awards (shared with A. Davidson, M. Bassan and R. Zamostiano). The Lily and Avraham Gildor Chair for the Investigation of Growth Factors.
2000	Best scientist award, the Israeli Society for Laboratory Studies
2000-2003	The Neufeld Grant Award for excellent BSF grant application, Health Science
2003	Excellent poster award The 6th International Symposium on VIP, PACAP and Related Peptides (with Inna Divinski, Hakone, Japan).
2003/4	Best student best paper, Brain Research (Dr. Albert Pinhasov, USA).
2003/2004	SAIA Doctoral Prize and Scholarship Fund for HIV and Parkinson's Diseases Research Tel Aviv University
2004/2005 -	Tel Aviv Univ. Dan David Scholarship, Shmuel Mandel
2005 -	Tel Aviv Univ. Buchmann Doctoral Scholarship Fund, Miri Holtser-Cochav
2006-2008	Jerusalem, Israel, Levi Eshkol Fellowship, Natalya Sheriev

2006-	Boaz Moav Prize for Develop. Biology, Switzerland Inst at TAU, Shmuel Mandel
2007-	Poster Award (Yan Jouroukhin) and Scholarship (Shmuel Mandel) ILMAR.
2008-	Tel Aviv University Vice President Prize for Innovative Research (University-Wide)

GRANTS

1982-1985	US-Israel-Binational Science Foundation, Jerusalem. "Vasoactive Intestinal Peptide, from Gene to Peptide" - \$60,000. Jerusalem, Israel.
1982-1984	Israel Academy of Sciences and Humanities "Control Mechanisms in the Expression of a Regulatory Peptide in the Nervous System" - \$20,000. Jerusalem, Israel.
1982-1986	Dysautonomia Foundation "Investigation of the Regulation of the Biosynthesis and Mechanism of NGF and NGF-like Factors" - \$40,000. New York, NY, USA.
1982-1989	The Leo and Julia Forschheimer Center for Molecular Genetics Grant. "The Molecular Genetics of Vasoactive Intestinal Peptide (VIP): Use of Recombinant DNA Technology to Study an Abundant regulatory peptide." - \$30,000. New York, NY, USA.
1983-1988	NIH (NINCDS) Grant. "Vasoactive Intestinal Peptide - From Gene to Peptide" - \$130,000. Bethesda, MD, USA.
1985	Weizmann Institute Center for Neuroscience Research Grant - \$1,000. Rehovot, Israel.
1985	Rockefeller-Weizmann Equipment Grant. "Steroid Hormone Regulation of the Hormone Transmitter Vasoactive Intestinal Peptide (VIP) Gene" - \$5,000.
1986-1989	Mrs. Cecelia Wilmers Grant for studies on Familial Dysautonomia - \$45,000. Geneva, Switzerland.
1986-1989	US-Israel-Binational Science Foundation. "Vasoactive Intestinal Peptide, from Gene to Peptide." - \$90,000. Jerusalem, Israel.
1987-1988	ETP grant. "Hormonal regulation of Vasoactive Intestinal PeptideExpression" -\$6,000. Strasbourg, France.
1987-1989	Israel Dysautonomia Foundation. "In Search of the Afflicted Gene in Dysautonomia"- \$10,000. Israel.
1987-1997	Industrial Grant on: Production of VIP Super-Active Analogues, for treatment of Impotence and Dementia. In cooperation with Dr. M. Fridkin. about \$2,400,000. With the possibility to extend. Japan.
1991-1992	Israel Cancer Research Foundation: "VIP in Cancer" \$15,000/year, for two years.
1992-1994	The Israeli National Fund for Research and Development, The Office for Science and Technology. "New neurotrophic Hormones" - \$10,000/first year, 20,000/ secondyear and third years.

1992-1995	I. Gozes, Ph.D. The US-Israel Binational Science Foundation, together with Dr. D.E.Brenneman (NIH, NICHD): Molecular characterization of activity dependent neurotrophic factor ADNF; \$30,500/year (for three years).
1993-1993	For the Faculty of Medicine, University support for equipment - by the vice president for research and development- \$4,500/ half a year.
1994-1994	University support for equipment - by the vice president for research and development-\$4,200/half a year.
1996-1997	Industrial support, \$200,000/year +\$150,000 (option fee, the first year) for developing an anti impotence drug (in collaboration with Prof. Mati Fridkin, Weizmann Inst. of Science).
1997-1999	Biological effects of VIP on Intrauterine growth retardation (The Israeli Ministry of Health) (\$11,905/year, two years) Biological Aspects of Novel Growth factors (Tel Aviv University fund) \$3,500
1997-1999	Industrial Support (\$225,000 in collaboration with Dr. D.E. Brenneman, NIH.
1997-2000	The US-Israel Binational Science Foundation, together with Dr. D.E. Brenneman (NIH, NICHD): Molecular characterization of activity dependent neurotrophic factors; \$38,500/year (for three years).
1998-1998	The Adams Super Center for Brain Studies- \$10,000, equipment grant.
1998-1998	The University fund- \$9,000, cancer research.
1999-2001	The Institute for the study of the aging (\$100,000/ first year; \$120,000/second year)
1999-2003	The Israel Science Foundation (about \$32,000/year for four years)
2000-2003	The US-Israel Binational Science Foundation, together with Dr. D.E. Brenneman (NIH, NICHD): \$34,000/year
2000-2001	The Sackler Faculty of Medicine (\$7000/year)
2001-2002	A Tel Aviv University Award (\$12,500/year)
2002-	A Tel Aviv University Cancer center Award (\$20,000/year)
2003-2011	Allon Therapeutics through Ramot (\$100,000-\$250,000/year) with the possiblity to extend
2003	Summer Neuropeptide Meeting
2003-2004	Teva Contract through Ramot (\$100,000/year)
2003-2004	The Mariana and George Saya grant, HIV and Parkinson (Tel Aviv Univ. \$5000)
2003-2004	Nofar, industrial grant through Ramot (~\$100,000/year –neuropeptides and sexual function, in collaboration with JnJ, Israel) – had to decline due to a Sabbatical conflict
2003-2007	The Israel Science Foundation (about \$32,000/year for four years)
2004-2008	The US-Israel Binational Science Foundation, together with Dr. Peng Y. Loh and Dr. Joanna Hill (NIH) (\sim \$32,000/year for four years)

Johnson and Johnson through Ramot (~\$20,000) 2005-

Nofar, industrial grant through Ramot (~\$100,000/year –inhibition of cancer growth by antisense oligodeoxynucleotides, in collaboration with Teva, Israel) 2006-

IsrALS (drug screening - \$20,000/eight months); A.M.N \$50,000/year (possibility for extension for 4 years) innovative neuroscience research – ADNP, ADNP2 and NAP 2008-

MEMBERSHIP AND LEADERSHIP IN PROFESSIONAL SOCIETIES

MEMBERSHI	AND DEADEROIN INTROLESSION IN SOCIETIES
1979-	Member of the Society of Neuroscience, USA.
1981-	Member of the New York Academy of Sciences (by invitation) Permanent Membership Awarded in 1985, USA.
1984-	Member of the Israeli Society for Endocrinology, Israel.
1987-	Member of the International Society of Developmental Neuroscience, USA.
1987-	Member of the Scientific Counsel of the Weizmann Institute of Science. Israel.
1987-	Editorial Board of The Journal of Molecular Neuroscience (Birkhauser Boston Inc. Publishers, as of 1992, published by Humana Press) USA.
1989-1992	Editorial Board of The New Biologist (Saunders Scientific Publications). USA.
1992-	Member-the Israeli Society for Clinical Biochemistry (appointed member of the fund for advancement of clinical chemistry in Israel - 1993).
1992-1997	Member-Scientific Committee of the Institute for Molecular Medicine, Sackler School of Medicine, Tel Aviv University.
1992-1996	Search and new appointments committee, Sackler School of Medicine, Tel Aviv University.
1993-	Member-The European Neuroendocrine Association.
1993-1996	Member-"Ramot-Tel Aviv University" Fund for Research.
1994-	Ad Hoc committee for absorption of scientists - new immigrants "The Ministry for Science and the Arts."
1995-	Member of the International Society for Neurochemistry.
1997-	Ad Hoc committee for prizes of excellency for the Biennial Doris Cecilia Levy Memorial Seminar in Brain Research.
	Member Tel Aviv University committee for Masters Degrees. (-2000)
	Member of the committee of the Israeli Neuroscience Society (-1999)
	International Committee for the determination of VIP and PACAP receptor nomenclature.
1998- 2002	Member of the Sackler Faculty of Medicine: 1. Research and development committee. 2. Graduate school committee.

1999-	Member of the Sackler Faculty pre-clinical appointment and promotion committee
1999-	Editor, The Journal of Molecular Neuroscience, Human Press.
2000-	Member, scientific advisory board: the Institute for the study of aging, NY, USA Executive guest editor: Current pharmaceutical Design Co-Chair- American Summer Neuropeptide Conference
2000-2003	Member: Clore Scholar Board
2002-	Editor-in-Chief - Journal of Molecular Neuroscience, Human Press.
2002-	Tel Aviv University Senate Committee for postdoctoral fellows Sackler Faculty of Medicine, Tel Aviv University, Grants Committee
2003-	Secretary General, the European Neuropeptide Club (ENC). Selection committee for Gotlieb Award (ISOA, NY, USA), VIP and Related Peptide, Scientific Advisory Committee
2004-	Editorial Board, American Journal of Alzheimer's Research and other Dementias (USA) International Advisory Committee, the VIP PACAP and Related Peptides Meeting Member, Winter Neuropeptides Steering Committee (USA)
2005-	Member, Scientific Advisory Committee: The United State Israel Binational Science Foundation Editorial Board, Peptides, Elsevier Press Editorial Board, Peptide Research and Therapeutics
2006-	Board member: Drug discovery, development & delivery for chronic neurodegenerative disease: a course for academic and biotechnology scientists- Alzheimer's Drug Discovery Foundations, New York (USA)
2007- 2008-	President-elect, the Israel Society for Neuroscience Editorial Board, International Journal of Peptides

STUDENTS IN THE WEIZMANN INSTITUTE OF SCIENCE

1982-1984	H. Schwartz, M.Sc. "Towards the Biosynthesis of VIP" (In cooperation with Dr. Fridkin)
1982-1985	M. Bodner, Ph.D. "Isolation and Characterization of the Human Gene Encoding Vasoactive Intestinal Polypeptide", (In cooperation with Dr. Mati Fridkin).
1986-1987	M. Fawzi, M.Sc. "The Neuropeptide VIP and it's Receptor."
1984-1989	R. Avidor, Ph.D. "In Situ Localization of Gene Expression: The Neuropeptides VIP and PHI-27.
1984-1989	E. Giladi, Ph.D. "Control Regulating the Expression of the Vasoactive Intestinal Polypeptide (VIP) Gene". (Awarded the Levi Eshkol Fellowship).
1987-1989	R. Levy Holtzman, M.Sc. " Synaptic Plasticity of VIP Gene Expression."

STUDENTS AT SACKLER SCHOOL OF MEDICINE, TEL-AVIV UNIVERSITY

1991-1995	A. Bardea (M.Sc. recommended distinction) "The physiology of VIP." Excellence Prize (Israeli Soc. for Clin. Biochemistry).
1990-1994	R. Glazer - (M.Sc. with distinction) "Is VIP an Steiner integral part of the biological clock in mammals" (Dean's list for excellent students), Best poster award and excellence prize, (Israeli Society for Clinical Biochemistry).
1990-1997	A. Davidson (Ph.D.) "Molecular Cloning of Activity-Dependent Neurotrophic
1992-	Factors." Excellence Prizes (Israeli Society for Clinical Biochemistry) M. Bassan (Ph.D. candidate) "Activity- Dependent Neurotrophic Factor, a possible family of growth factors" (Best poster prize the Israeli Society for Clinical Biochemistry).
1992-	Y. Nesher (M.Sc. Candidate) "VIP receptors in cancer".
1994- 2000	R. Zamostiano (Ph.D. awarded 2001) "Neuronal apoptosis" (Best poster prize the Israeli Society for Clinical Biochemistry).
1994-1995	M. Bechar (M.Sc. recommended distinction) "VIP and developmental deficiencies in ApoE-mice".
1994-1995	O. Ashur-Fabian (M.Sc. with distinction) "VIP in Alzheimer's disease". Dean's list of excellence.
1994-2000	R. Granoth (Ph.D. with Prof. M. Fridkin, Weizmann Institute of Science) "VIP and skin in health and disease."
1995-2001	O. Ashur-Fabian (Ph.D.) "Mechanism of VIP activity". Dean's list of excellence.
1997-1998	A. Pinhasov (M.Sc. excellent) "VIP gene expression in health and disease".
1998-2000	A. Sigalov (M.Sc.) "High blood pressure, growth retardation and stroke"
1998-2001	J. Romano (M.Sc.) "VIP/ADNP and head trauma".
1999-2002	I. Volih (M.Sc.) "Processing of hsp60".
1999-2005	S. Furman (Ph.D.) "Biological activity and processing of activity-dependent neuroprotective protein (ADNP)".
2000-2002	Y. Segal-Ruder (M.Sc. Excellent) "Neuroprotection mechanisms: the effect of oxidative stress on the expression of VIP, PACAP peptides and the receptors VPAC1 and VPAC2 in astroglia"
2001-2005	R. Zaltzman (M.D./Ph.D.) "The involvement of ADNP and its derivative NAP in injury to
2000-2003	the central nervous system". M. Michlin (M.Sc.) "Glial proteins that mediate the activity of neuropeptides from the VIP family."
	family" M. Leiderman (M.Sc., Excellent oral examination) "The mechanism of SNV action: in search for SNV binding proteins and its influence on gene expression in the NF kappa B signaling pathway."
2000-2006	I. Piltzer (Ph.D.) "Isolation of the neuronal survival mediating VIP receptor"I. Divinski (Ph.D.) "Proteins that mediate NAP neuroprotection".D. Dangoor (Ph.D., with Prof. M. Fridkin, Weizmann Institute of Science)

"VIP; Design of novel analogs and sexual functions"

2002- I. Vulih-Shultzman (Ph.D. candidate)

"Partial deficiency in ADNP".

2003- Shmuel Mendel (Direct Ph.D. candidate)

2003-2005 "Characterization of Activity-dependent neuroprotective protein"

Irit Spivak-Pohis (M.Sc.) "ADNP processing"

Karin Vered (M.Sc.) Tau phosphorylation in experimental encephalopathy

Michal Kukshnir (M.Sc. Novel gene KIAA 0863 Alexander Kryvoshey (M.Sc.) Partial deletion/ADNP

Miri Holtser-Cochav (M.Sc.) "Characterization of NAP binding to tubulin: a unique

mechanism of neuroprotectoin"

2005 - Ilona Zemlyak (Ph.D. candidate) in collaborations with R. Sapolsky, Stanford University

"NAP, ADNP and epilepsy.

Efrat Dresner (M.D./Ph.D. candidate)

"The relationship between ADNP and KIA 0863"

Natalia Shiryaev (Ph.D. candidate)

"Novel growth factors in animal models of Alzheimer's disease and tauopathies"

2004 Saar Oz (M.Sc. candidate) "Molecular modelling and docking simulation of NAP &

Tubulin in order to identify and understand the binding mode between them."

2005 Inbal Michaelovich, (M.Sc. candidate) "Cancer treatment by ADNP antisense"

Maya Maor, (M.Sc. candidate) "Characterization of NAP binding to tubulin and of NAP

binding to tubulin"

Shmuel Goldberg, (Ph.D. direct candidate) "Learning behavior Parp Inhibition and lack

of special memory creation"

Tal Shprung, (M.Sc. candidate) "NAP protection from taxol neuropathy"

2006- Yan Jouroukhin (M.Sc. candidate) "ALS neuroprtection"

2007- Avia Merlander (Ph.D. candidate) "Protection in diabetes neuropathy"

Shumert Ben-Yosef (M.Sc. candidate- Direct Ph.D. candidate 2008-) "tubulin and learning"

2008- Anat Idan (Ph.D. candidate) "Neuropathy and Cognition"

Yulie Schirer (M.Sc. candidate) "Taoupathy"

SABBATICAL FELLOWS PHYSICIANS (M.Sc. STUDIES) AND VISITORS

D. Wurtman (Harvard University), summer student.

Dr. C. Dutlow (University of Cape Town, South Africa)

1984 E. Bril (Summer student)

1985 Prof. Maynard Makman (Albert EinsteinCollege of Medicine)

Iris Geffen (Albert Einstein College of Medicine)

1986 Dr. William Rostene (INSERM, INSERM Fellowship)

Dr. Pinhas Shachter (Sheba Medical Center, Tel Hashomer, Israel).

1987 Dr. Hanan Goldman (Hadassah Medical School, Jerusalem, Israel).

Prof. Bruce McEwen (Rockefeller University)

Dr. Frank Baldino (President, Cephalon Inc.)

1989, 1992, 1993,1994, 1995,

1997,2000,2001

Dr. Douglas E. Brenneman (NICHD, NIH)

1996-

Dr. Haim Bassan (Tel Aviv Medical Center)

1997-

Dr. Albert Levi (Rabin Medical Center)

1998-

Dr: Edgar Gelber (Rabin Medical Center)

1999-

Dr. Yafim Rabinovitz (Rabin Medical Center)

2000-

Dr. Eitan Heldenberg (Sheba Medical Center)

Dr. Michael Rothstein (Tel Aviv Medical Center)

Roy Zaltzman (MD student) Roy Alcalay (MD student)

2001-

Dr. Avron D. Spier (Scripps Research Institute) Andrew M. Rosensweig (Summer student, MIT)

2001-2003

Dr. Roy Alcalay (MD student)

2002-

Dr. Avron D. Spier (guest scientist)

2003-

Amit Meshi (MD student)

2006-

Dana Yelin (MD student)

Post Doctoral Fellows

1991-1995

Dr. Gila Lilling- Kolton Foundation

1992-1994

Dr. Sergei Zhukovsky

1995-

Dr. Orly Perl

1998-2000

The late Dr. Ruth Steingart-Koret Foundation

Resident scientists

1995-1995

Dr. Zila Blatt

1995-

Dr. Eliezer Giladi

2001-2005

The late Dr. Ruth Steingart

2002-2003

Dr. Zipora Pittel (sabbatical)

2005-2006

Dr. Albert Pinhasov (consultant)

2006-2007

Prof. Nava Naveh (sabbatical)

2007-

Dr. Sharon Furman-Assaf

LIST OF PUBLICATIONS:

A. ARTICLES:

- 1. I. Gozes, H. Schmitt and U.Z. Littauer. Translation in vitro of rat brain messenger RNA coding for tubulin and actin.Proc. Natl. Acad. Sci. USA 72, 701-705, 1975.
- 2. M.D. Walker, I. Gozes, A.M. Kaye, N. Reiss and U.Z. Littauer. The estrogen induced protein: Quantitation by autoradiography of polyacrylamide gels. J. Steroid Biochem. 7, 1083-1085, 1976.
- 3. U.Z. Littauer, H. Schmitt and I. Gozes. Properties and synthesis of tubulin in neuroblastoma cells. J. Natl. Cancer Inst. 57, 647-651, 1976.
- 4. H. Schmitt, I. Gozes and U.Z. Littauer. Decrease in levels and rates of synthesis of tubulin and actin in developing rat brain. Brain Res. 121, 327-342, 1977.
- 5. I. Gozes, U.Z. Littauer, B. Geiger and S. Fuchs. Immunochemical determination of tubulin. FEBS Lett. 73, 109-114, 1977.
- 6. I. Gozes, M.D. Walker, A.M. Kaye and U.Z. Littauer. Synthesis of tubulin and actin by neuronal and glial nuclear preparations from developing rat brain. J. Biol. Chem. 252, 1819-1825, 1977.
- 7. I. Gozes and U.Z. Littauer. Tubulin microheterogeneity increases with rat brain maturation. Nature 276, 411-413,1978.
- 8. I. Gozes, and C. Richter-Landsberg. Identification of tubulin associated with rat brain myelin. FEBS Lett. 95 169-172, 1978.
- 9. R. Salomon, M. Bar-Yosef, H. Soreq, I. Gozes and U.Z.Littauer. Translation in vitro of Carnation Mottle Virus RNA: Regulatory function of the 3'-region. Virology 90 288-298, 1978.
- 10. I. Gozes and U.Z. Littauer. The alpha-subunit of tubulinis preferentially associated with brain, presynaptic membranes. FEBS Lett. 99, 86-90, 1979.
- 11. I. Gozes, D. Saya and U.Z. Littauer. Tubulin microheterogeneity in neuroblastoma and glioma cells differs from that of brain. Brain Res. 171,171-175, 1979.
- 12. M.D. Walker, V. Negreanu, I. Gozes and A.M. Kaye. Identification the estrogen induced protein in uterus and brain of untreated immature rats. FEBS Lett. 98 187-191, 1979.
- 13. I. Gozes, A. de Baetselier and U.Z. Littauer. Translation of rat brain mRNA coding for multiple tubulin species. Eur. J. Biochem. 103, 13-20, 1980.
- 14. N. Zisapel, M. Levi and I. Gozes. Tubulin an integral protein of mammalian synaptic vesicle membranes. J. Neurochem. 34, 26-32, 1980.
- 15. R. Hofstein, M. Hershkowitz, I. Gozes and D. Samuel. Characterization and phosphorylation of an actin-likeprotein in synaptosomal membranes. Biochem. Biophys. Acta. 624, 153-162, 1980.

- 16. I. Gozes, B.L. Cronin and M.A. Moskowitz. Protein synthesis in rat brain microvessels decreases with aging. J. Neurochem. 36, 1311-1315, 1981.
- 17. I. Gozes and K.J. Sweadner. Multiple forms of tubulinare expressed by a single neuron. Nature 294, 477-480,1981.
- 18. I. Gozes and C.J. Barnstable. Monoclonal antibodies that recognize discrete forms of tubulin. Proc. Natl. Acad. Sci. USA 79, 2579-2583, 1982.
- 19. I. Gozes, D.T. O'Connor and F.E. Bloom. A possible high molecular weight precursor to vasoactive intestinal polypeptide sequestered into pheochromocytoma chromaffin granules. Regulatory Peptides 6, 111-119, 1983.
- 20. Y. Gozes, M.A. Moskowitz, T.B. Strom and I. Gozes. Conditioned media from activated lymphocytes maintain sympathetic neurons in culture. Developmental Brain Research, 6, 93-97,1983.
- 21. I. Gozes, R.J. Milner, F.-T. Liu, E. Johnson, E.L.F.Battenberg, D. Katz and F.E. Bloom. Monoclonal antibodiesagainst vasoactive intestinal polypeptide: Studies of structure and related antigens. J. Neurochem 41, 549-556,1983.
- 22. I. Gozes, M. Bodner, Y. Shani and M. Fridkin. Detection of mRNAs containing regulatory peptide sequences using synthetic oligodeoxynucleotide probes. J. Cell Biochem. 26, 147-156, 1984.
- 23. I. Gozes, M. Bodner, H. Schwartz, Y. Shani and M.Fridkin. Studies toward the biosynthesis of vasoactive intestinal peptide (VIP) Peptides, 5, 161-166, 1984.
- 24. M. Bodner, M. Fridkin and I. Gozes. Coding sequences for vasoactive intestinal peptide and PHM-27 peptide are located on two adjacent exons in the human genome. Proc. Natl. Acad. Sci. USA, 82, 3548-3551, 1985.
- 25. H. Werner, Y. Koch, M. Fridkin, J. Fahrenkrug and I.Gozes. High levels of vasoactive intestinal peptide in human milk. Biochem. Biophys. Res. Commun. 133, 228-232, 1985.
- 26. I. Gozes, M. Bodner, Y. Shani and M. Fridkin. Structure and expression of the vasoactive intestinal peptide (VIP) gene in a human tumor. Peptides 7, 1-6, 1986.
- 27. I. Gozes and A. Tsafriri. Detection of VIP-encoding mRNA in the rat ovaries. Endocrinology 119, 2606-2610, 1986.
- 28. I. Gozes and Y. Shani. Hypothalamic VIP-mRNA is increased in lactating rats. Endocrinology 119, 2497-2501, 1986.
- 29. I. Gozes, R. Avidor, D. Katznelson, Y. Yahav, C. Croce and K. Huebner. The gene encoding vasoactive intestinal peptide is located on human chromosome 6p21-6qter. Human Genetics 75, 41-44, 1987.
- 30. I. Gozes, E. Giladi and Y. Shani. VIP-gene expression: Putative mechanism of information storage at the RNAlevel. J. Neurochem. 48, 1136-1141, 1987.
- 31. I. Gozes, Y. Shani and W.H. Rostene. Developmental expression of the VIP-gene in brain and intestine. Mol.Brain Res. 2, 137-148, 1987.

- 32. I. Gozes, H. Nakai, M. Byers, R. Avidor, Y. Weinstein, Y.Shani and T.B. Shows. Sequential expression in the nervous system of the VIP and c-myb genes located on the human chromosomal region 6q24. Somatic Cell and Mol. Gen. 13(4) 305-313, 1987.
- 33. B.D. Boss, I. Gozes and W.M. Cowan. The survival of dentate gyrus neurons in dissociated cultures. Dev. Brain Res. 36, 199-218, 1987.
- 34. I. Gozes, P. Shachter, Y. Shani and E. Giladi. Vasoactive intestinal peptide gene expression from embryos to aging rats. Neuroendocrinology, 47, 27-31, 1988.
- 35. J.P. Card, S. Fitzpatrick-McElligott, I. Gozes and F.Baldino, Jr. Localization of vasopressin, somatostatin and VIP messenger RNA in the rat suprachiasmatic nucleus. Cell and Tissue Res. 252, 307-315,1988.
- 36. H. Werner, Y. Koch, F. Baldino Jr. and I. Gozes. Steroid regulation of somatostatin mRNA in the rat hypothalamus. J. Biol. Chem. 263, 7666-7671, 1988.
- 37. F. Baldino, Jr., S. Fitzpatrick-McElligot, T.M. O'Kane andl. Gozes. Hormonal regulation of somatostatin messenger RNA. Synapse 2, 317-325, 1988.
- 38. I. Gozes, H. Werner, M.A.A. Fawzi, Y. Shani, M. Fridkin and Y. Koch. Estrogen regulation of vasoactive intestinal peptide mRNA in the rat hypothalamus. J. Molec. Neurosci. 1 55-61, 1989.
- 39. I. Gozes, R. Avidor, A. Biegon and F. Baldino, Jr. Lactation elevates vasoactive intestinal peptide messenger ribonucleic in rat suprachiasmatic nucleus. Endocrinology, 124, 181-186, 1989.
- 40. F. Baldino, S. Fitzpatrick-McElligott, I. Gozes and J.P Card. Localization of VIP and PHI-27 messenger RNA in rat thalamic and cortical neurons. J. Mol. Neurosci. 1, 199-207, 1989.
- 41. R. Levy Holtzman, R. Malach and I. Gozes. Disruption of the optic pathway during development affects vasoactive intestinal peptide mRNA expression. The New Biologist 1, 215-221, 1989.
- 42. I. Gozes, E. Meltzer, S. Rubinrout, D.E. Brenneman and M.Fridkin. Vasoactive intestinal peptide potentiate sexual behavior: Inhibition by novel antagonist. Endocrinology 125, 2945-2949, 1989.
- 43. I. Gozes, Y. Shani, B. Liu and J.P. Burbach. Diurnal variation in vasoactive intestinal peptide messenger RNA in the suprachiasmatic nucleus of the rat. Neuroscience Research Communications 5, 83-86, 1989.
- 44. I. Gozes and D.E. Brenneman. VIP molecular biology and Neurobiological function. Molecular Neurobiology, 3, 201-236, 1989.
- 45. R. Avidor, R. Eilam, R. Malach and I Gozes. VIP-mRNA is increased in hypertensive rats. Brain Res. 503, 304-307, 1989.
- 46. E. Giladi, Y. Shani and I. Gozes. The complete structure of the rat VIP-gene. Mol. Brain Res. 7, 261-267, 1990.

- 47. D.E. Brenneman, S.K. McCune and I. Gozes. Acquired immune deficiency syndrome and the developing nervous system. International Review of Neurobiology, 32, 305-353, 1990.
- 48. Y. Gozes, D.E. Brenneman, M. Fridkin, R. Asofsky and I.Gozes. A VIP antagonist distinguishes VIP receptors on spinal cord cells and lymphocytes. Brain Res. 540, 319-321, 1991.
- 49. J.M. Hill, I. Gozes, J.L. Hill, M. Fridkin and D.E.Brenneman. Vasoactive intestinal peptide antagonist retards the development of neonatal behaviors in the rat. Peptides, 12, 187-192, 1991.
- 50. I. Gozes, S.K. McCune, L. Jacobson, D. Warren, T.W. Moody, M. Fridkin and D.E. Brenneman. An antagonist to vasoactive intestinal peptide: effects on cellular functions in the central nervous system. J. Pharmacol. and Exp. Therap. 257,959-966, 1991.
- 51. D.V. Agoston, L.E. Eiden, D.E. Brenneman and I. Gozes. Spontaneous electrical activity regulates vasoactive intestinal peptide expression in dissociated spinal cord cell cultures. Mol. Brain Res. 10, 235-240, 1991.
- 52. J.R. Glowa, L.V. Panlilio, D.E. Brenneman, I. Gozes, M.Fridkin and J.M. Hill. Learning impairment following intracerebral administration of the HIV envelope protein gp120 or a VIP antagonist. Brain Res. 570, 49-53, 1992.
- 53. D.E. Brenneman, M. Schultzberg, T. Bartfai and I. Gozes. Cytokine regulation of neuronal survival. J. Neurochem. 58, 454-460, 1992.
- 54. I. Gozes and M. Fridkin. A fatty neuropeptide: potential drug for noninvasive impotence treatment in a rat model. J. Clinical Investigation. 90, 810-814, 1992.
- 55. M. Dussaillant, A. Sarrieau, I. Gozes, A. Berod and W.Rostene. Distribution of cells expressing vasoactive intestinal peptide/peptide histidine isoleucine-amide precursor messenger RNA in the rat brain. Neuroscience. 50, 519-530, 1992.
- 56. I. Gozes and D.E. Brenneman. Neuropeptides as growth and differentiation factors in general and VIP in particular. J.Molecular Neurosci. 4, 1-9, 1993.
- 57. P. Gressens, J.M. Hill, I. Gozes, M. Fridkin and D.E.Brenneman. Growth factor function of vasoactive intestinal peptide in whole cultured mouse embryos. Nature, 362, 155-158, 1993.
- 58. T.W. Moody, F. Zia, M. Draoui, D.E. Brenneman, M.Fridkin, A. Davidson and I. Gozes. A VIP antagonist inhibits non-small cell lung cancer growth. Proc. Natl. Acad. Sci.USA, 90, 4345-4349, 1993.
- 59. Y. Wollman, G. Lilling, M.N. Goldstein, M. Fridkin and I.Gozes. Vasoactive intestinal peptide: a growth promoter in neuroblastoma cells. Brain Research, 624, 339-341, 1993.
- 60. I. Gozes, J. Glowa, D.E. Brenneman, S.K. McCune, E. Lee and H. Westphal. Learning and sexual deficiencies in transgenic mice carrying a chimeric vasoactive intestinal peptide gene. J. Mol. Neurosci., 4,185-193,1993.

- 61. I. Gozes, R. Avidor, E. Giladi, Y. Shani, B.S. McEwen, M.Dussaillant and W.H. Rostene. Adrenalectomy decreases vasoactive intestinal peptide-mRNA levels in the rat suprachiasmatic nucleus. Neurosci. Lett. 167,24-28,1994.
- 62. R. Glazer and I. Gozes. Diurnal oscillation in vasoactive intestinal peptide gene expression independent of environmental light entraining. Brain Res. 644,164-168,1994.
- 63. I. Gozes, A. Reshef, D. Salah, S. Rubinrout and M. Fridkin. Stearyl-Norleucine-VIP a novel VIP analogue for noninvasive impotence treatment. Endocrinology, 134,2121-2125,1994.
- 64. P. Gressens, J.M. Hill, B. Paindaveine, I. Gozes, M.Fridkin and D.E. Brenneman. Severe microcephally induced byblockade of vasoactive intestinal peptide function in the primitive neuroepithelium of the mouse. J. Clin. Invest. 94, 2020-2027, 1994.
- 65. M. Sone, D.M. Smith, M.A. Ghatei, I. Gozes, D.E. Brenneman, M. Fridkin, and S.R. Bloom. Pituitary adenylate cyclase activating polypeptide (PACAP)/vasoactive intestinal peptide (VIP) receptor subtypes in rat tissues: investigation of receptor binding and molecular identification by chemical cross linking. Biomedical Research, 15, 145-153, 1994.
- 66. I. Gozes, G. Lilling, R. Glazer, A. Ticher, I.E. Ashkenazi, A. Davidson, S. Rubinraut, M. Fridkin and D.E. Brenneman. Superactive lipophilic peptides discriminate multiple VIP receptors. J. Pharmacol. Exper. Therap. 273, 161-167, 1995.
- 67. D.E. Brenneman, J.M. Hill, G.W. Glazner, I.Gozes and T.W.Phillips. Interleukin-1 alpha and vasoactive intestinal peptide: enigmatic regulation of neuronal survival. Int. J. Dev. Neurosci. 13, 187-200, 1995.
- 68. G. Lilling, Y. Wollman, M.N. Goldstein, S. Rubinraut, M.Fridkin, D.E. Brenneman and I. Gozes. Inhibition of Human Neuroblastoma Growth by a Specific VIP Antagonist. J.Molec. Neurosci. 5, 231-239, 1995.
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Presentations/abstracts (January 2004 -)

- January 2004
- The 25th Winter Neuropeptide Conference, Breckenridge CO, USA, January 31- February 3, 2004

Committee member, session chair: Translational Research: Peptides from the lab bench to the clinic. Lecture: NAP: Discovery and development of an eight amino acid neuroprotective peptide drug candidate.

February 2004

 International Congress of Biological Psychiatry, Sydney Convention & Exhibition Centre, Sydney, Australia, February 9 – 13, 2004

Lecture: Intranasal administration of NAP, a neuroprotective peptide Decreases Anxiety-Like Behavior in Aging Mice in the Elevated Plus Maze.

March 2004

• Intranasal CNS Drug Delivery (Management Forum Ltd), London, England, March 29-30, Lecture.

May 2004

 NEUROPEPTIDES 2004 XIV EUROPEAN NEUROPEPTIDES CLUB MEETING ALICANTE, Spain, May 9-12, 2004, Secretary of the Club, Session Chair, lecture: From VIP and PACAP to ADNP and NAP: new horizons in drug development and neuroprotection.

Publication: Aracil A, Belmonte C, Calo G, Gallar J, Gozes I, Hoyer D, Patachini R, Schmidt RF, Zimmermann M. Proceedings of Neuropeptides 2004, the XIV European Neuropeptides Club meeting. Neuropeptides. 2004 Dec;38(6):369-71.

July 2004

- Summer Neuropeptide Meeting, Miami Beach, Florida, July 5-9. Meeting Co-Chair, Session Chair and Lecture: Publication: Gozes I, Brenneman DE. Summer Neuropeptide Conference: The 14th Annual Meeting of the Summer Neuropeptide Conference, Miami Beach, Florida, USA, July 5-9, 2004. Neuropeptides 39 (2005) 29-33 (all the meeting abstracts: 35-65, including:
- Gozes, S. Mandel, I. Divinski, D. E. Brenneman, A. D. Spier.
 From homeobox proteins to neuroprotection: potential new therapeutics. Page 45.
- R. A. Steingart, I. Gozes. Recombinant activity-dependent neuroprotective protein protects pheochromocytoma cells against oxidative stress. Page 64.
- I. Vulih, A. Pinhasov, D. E. Brenneman, N. Grigoriadis, I. Gozes. Growth restriction and learning deficits in male mice harboring one copy of the gene encoding activity-dependent neuroprotective protein. Pages 63-64.
- The 9th International Conference on Alzheimer's Disease and Related Disorders, July 17-22, Pennsylvania Convention Center, Philadelphia. Hot Topics poster presenter.

August 2004

Peptide receptors, July 31-August 4, Montreal, Canada.
 Lecture: Toward the isolation and characterization of the VIP receptors that mediate neuronal survival.

September 2004

• 12th International Congress of Endocrinology, August 31-September 4, Lisbon, Portugal.

Lecture: Peptides and cellular protection.

 3rd International and 28th European Peptides Symposium, September 5-10, Prague, Czech Republic.

Lecture: Novel neuroprotective peptides: from genes to behavior and potential therapeutics.

November 2004

- Society for Neuroscience Meeting, November 12-16, San Diego, CA, USA, oral presentation:
- I. Gozes, I. Divinski, I. Vulih, S. Furman, A. Pinhasov, Z. Pitel, N. Grigoriadis, D.E. Brenneman. MICROTUBULE-RELATED NEUROPROTECTION: ACTIVITY-DEPENDENT NEUROPROTECTIVE PROTEIN (ADNP), NAP, TUBULIN, AND TAU Program No. 485.3. 2004 Abstract Viewer/Itinerary Planner. Washington, DC: Society for Neuroscience, 2004. Online.
- Israel Society for Neuroscience, Eilat, November 28-30
 Abstracts- Neural Plasticity: Abstracts of the 13th Annual Meeting of the Israel Society for Neuroscience, Vol. 12, No. 1:
 - Page 13: I. Divinski, L. Mittelman and I. Gozes. NAP binding to tubulin mediates cell survival.
 - Page 27: M. Holtser, K. Vered, S. Furman, S. Mandel, I. Gozes. Immunochemical recognition of activity dependent neuroprotective protein (ADNP) and the active peptide NAP.
 - Page 33: M. Kushnir, S. Mandel, I. Gozes. Characterization of a novel brain protein- KIAA0863.
 - Page 39: S. Mandel, N. Amariglio, J. Jacob, G. Rechavi, I. Vulih, I. Gozes. Activity-dependent neuroprotective protein regulates neurogenin 1: a novel gene important for neurogenesis.
 - Page 47: I. Pilzer, R.A. Steingart, D. Dangoor, S. Rubinrot, M. Fridkin, D.E. Brenneman and I. Gozes. Toward the isolation and characterization of the VIP receptor that mediates neuronal survival.
 - Page 59: R.A. Steingart, I. Gozes. The recombinant fusion protein VP-22-activity-dependent neuroprotective protein protects pheochrmocytoma cells against oxidative stress.
 - Page 62: I. Vulih, A. Pinhasov, D.E. Brenneman, N. Grigoriadis, I. Gozes. Learning deficits and neuronal degeneration in male mice harboring one copy of the gene encoding activity-dependent neuroprotective protein (ADNP).

March 2005

- 7th International Conference on Alzheimer's and Parkinson's Disease, Sorrento, Italy, March 9-13
- Gozes ¹, I. Divinski ¹, I. Shultzman ¹, R.A. Steingart ¹ NEUROPROTECTION THROUGH MODULATION OF MICROTUBULE DYNAMICS: TOWARD CLINICAL DEVELOPMENT IN ALZHEIMER'S DISEASE

April 2005

 EUROPEAN NEUROPEPTIDES CLUB 2005 XV EUROPEAN NEUROPEPTIDES CLUB MEETING Riga, Latvia, May 19-21, 2004, Secretary of the Club, Session Chair, lecture: I. Gozes, I. Divinski, M. Holtser-Cochav. Neuroprotection: peptides downstream from VIP.

November 2005

 I. Gozes, I. Divinski, M. Holtser-Cochav. A NEUROPROTECTIVE DRUG CANDIDATE (AL-108 = NAP)

AND RELATED COMPOUNDS INTERACT WITH TUBULIN THROUGH A TAXOL-ASSOCIATED SITE Program No. 339.4. 2005 Abstract Viewer/Itinerary Planner. Washington, DC: Society for Neuroscience, 2005. Online.

December 2005

The Isreli Society for Neuroscience: Reviews in the neurosciences: Vol16, Suppl 1, 2005

- S31- Holtser- Cochav M. and Gozes I. NAP, a neuroprotective peptide, interacts specifically with brain beta III tubulin.
- S16- Divinski I and Gozes I. NAP, a femtomolar-acting peptide, interacts with tubulin and provides neuroprotection
- S25- Gozes I. and Giladi E. From the activity-dependent neuroprotective gene family to NAP toward clinical development
- S39- Kushnir M., Gozes I. Molecular characterization of the novel KIAA0863 gene
- S44- Mandel S., Gozes I. ADNP, retinoic acid and HP: toward the understanding ADNP actions
- S5-Pilzer I., Gozes I. A splice variant of PAC receptor mediates VIP protective activity on non-neuronal cells through cGMP
- S6-Spivak Pohis I., Mandel S., Loh YP. and Gozes I. Constitutive and regulated secretion of activity-dependent neuroprotective protein (ADNP)-like protein sequences

S66-Vulih-Shultsman I., Pinhasov A., Kryvoshey A. and Gozes I. ADNP is essential for brain function

January 28-31, 2006

27th Annual Winter Neuropeptide Meeting

Breckenridge, CO

Invited lecture

March 28-29, 2006

Strategic Research Institute's "CNS Diseases Congress: Alzheimer's Disease Track"

Cambridge, MA

Invited lecture

March 29-30, 2006

Management Forum on Nasal Delivery

London, UK

Invited lecture

May 29-31, 2006

Biomed Israel 2006

Jerusalem, Israel

Lecture

June 21-22, 2006

13th Tel Aviv University Alzheimer's Disease Conference

Tel Aviv, Israel

Lecture

June 28 - July 1, 2006

Summer Neuropeptide Meeting

Miami, FL

Invited lecture/Meeting Co-Chair (President)

July 9-14, 2006

Proprotein Processing, Trafficking & Secretion Gordon Research Conference, New London, NH

Invited lecture

July 15-20, 2006

10th International Conference on Alzheimer's Disease and Related Disorders,

Madrid, Spain

Invited lecture

October 12-13,2006

7th International Conference on Alzheimer's Disease Drug Discovery

New York, NY

Presentation, Advisory Board

October 14-18, 2006

Neuroscience Meeting USA

Atlanta, GA

Poster presentation

December 3-5, 2006

Israel Society for Neuroscience (ISFN)

Eilat, Israel

Invited lecture + poster presentations

February 5-6, 2007

Drug Discovery, Development & Delivery for Chronic Neurodegenerative Disease: A Course for Academic and Biotechnology Scientists.

New York, NY

Presentation, Advisory Board

March 1, 2007

Heart and Brain - TAU

March 14-18, 2007

AD/PD 2007, Salzburg, Austria

April 19 - 23, 2007

ENC 2007

European Neuropeptide Club

Annual Meeting

Santorini Island, Greece

Secretary General

Drug Discovery, Development & Delivery for Chronic Neurodegenerative

Disease

February 5-6, 2007

New York, NY

Presentation, Advisory Board

Heart and Brain

March 1, 2007

Tel Aviv University, Israel

Presentation

AD/PD 2007

March 14-18, 2007

Salzburg, Austria

Presentation

European Neuropeptide Club 2007

April 19-23, 2007

Annual Meeting

Santorini Island, Greece

Presentation

IUBMB Conference

May 21-25, 2007

Salvador de Bahia, Brazil

Presentation

The 14th Tel Aviv University Alzheimer's Disease Conference

June 13-14, 2007

Tel Aviv, Israel

Presentation

VIP/PACAP Meeting

September 3-7, 2007

Vermont, USA

Presentation/Meeting Co-Chair

Summer Neuropeptide Meeting

September 8 – 9, 2007

Vermont, USA

Presentation/Meeting Co-Chair (President)

SFN

November 3-7, 2007

San Diego, CA

Presentation

Adams Super Center satellite to the ISFN meeting

November 23-24, 2007

Eilat, Israel

Presentation

ISFN

November 25-27, 2007

Eilat, Israel

Presentation

The 4th International Winter-Conference on Alzheimer's Disease

December 7-10, 2007

Zürs, Austria

Presentation

2008 abstracts: ENC joint meeting, Ferrara, Italy, The 2nd Joint US-Israeli-Palestinian Brain Conference Jerusalem, ICAD Chicago, IL, USA(2); Drug Discovery & Development of Innovative Therapeutics, Boston, MA, USA

EXHIBIT H

NAP, a Peptide Derived from the Activity-Dependent Neuroprotective Protein, Modulates Macrophage Function

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RAFAEL FERNANDEZ-MONTESINOS,^c JUAN LUIS HERRERA,^c
ILLANA GOZES,^b IRUN R. COHEN,^a AND DAVID POZO^{a,c}

ABSTRACT: NAP is an eight-amino acid neuroprotective peptide NAPVSIPQ; it is the smallest active element derived from the recently cloned activity-dependent neuroprotective protein (ADNP). NAP readily enters the brain from the blood. It will be important to learn whether NAP, in addition to its neuroprotective activity, also might influence immune-mediated inflammation. Here, we report that: (a) macrophages express ADNP; (b) expression of ADNP in macrophages responds to VIP; and (c) NAP downregulates the key inflammatory cytokines tumor necrosis factor (TNF- α), interleukin-16 (IL-16), and IL-12 in macrophages. These findings indicate that ADNP/NAP can play an important role in immune regulation as well as in neuroprotection, which may be mutually related processes.

KEYWORDS: vasoactive intestinal polypeptide (VIP); activity-dependent neuroprotective protein (ADNP); macrophages; neuroimmunology; gene expression

INTRODUCTION

Neurotrophic proteins and neuropeptides have important regulatory functions and are a focus of intensive research in rational drug design.^{1,2} Previous

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studies have identified several components by sequential chromatographic methods within the neurotrophic milieu produced by astroglia, activity-dependent neurotrophic factor (ADNF) being one of the most potent.³ The active peptide fragment of ADNF is ADNF-14 (VLGGGSALLRSIPA)³; ADNF-9 (SALLRSIPA), a shorter C-terminal peptide, retains full biological activity.⁴ Antibodies to ADNF-14 or to ADNF-9 were used to identify the activity-dependent neuroprotective protein (ADNP), and the cDNA has been cloned from mouse neuroglial cells and human fetal brain.^{5,6} Structure/activity screening of several peptides derived from ADNP identified a potent octapeptide, NAP (NAPVSIPQ).⁵ NAP has a greater *in vivo* neuroprotective efficacy than ADNF-9.^{5,7,8}

The neuroprotective activities of NAP have been studied in a wide variety of systems. NAP induces neuroprotection against the β -amyloid peptide's toxicity involved in the onset of Alzheimer's disease, ^{5,9,10} oxidative stress, ¹¹ NMDA excitotoxicity, ⁵ tumor necrosis factor- α (TNF- α) toxicity, ¹² transient glucose deprivation, ⁹ dopamine toxicity, and decreased glutathione. ¹³ NAP's biological properties related to neuroprotection have been demonstrated in *in vivo* models of closed head injury, fetal alcohol syndrome, and stroke^{7,14} and it also been involved in neurodevelopment. ¹⁵ NAP is under phase I clinical trials in the United States.

Neuropeptides and neurotrophic proteins perform a broad array of seemingly unrelated functions. Vasoactive intestinal polypeptide (VIP), for example, promotes neuronal survival, 16,17 but is also a potent immunomodulator 18,19 and is under clinical trials. Remarkably, VIP inhibits the acute inflammatory response that follows spinal cord injury²⁰ and prevents activated microglia-induced neurodegeneration under inflammatory conditions²¹ while increasing the synthesis of the NAP-containing protein ADNP in astroglia. 5 Given the breath of NAP's neuroprotective activities and the fact that ADNP is a VIP-responsive gene, we were interested to examine the direct consequences of NAP on the immune system. The present article shows for the first time direct effects of NAP on the macrophage, a cell with a critical role in the initiation and coordination of the immune response. Mindful that VIP acts on activated macrophages as a potent, endogenous anti-inflammatory neuropeptide and that ADNP is a VIP-responsive gene, the current study was performed to investigate whether ADNP mRNA expression can be detected in a mouse macrophage cell line and whether VIP is able to increase the steady-state levels ADNP mRNA.

MATERIAL AND METHODS

Synthetic VIP was purchased from Calbiochem-Novabiochem (Laufelfingen, Switzerland). NAP was used as before.²² The mouse macrophage cell line RAW 264.7 was obtained from the American Type Tissue Collection (Rockville, MD). These cells were maintained in RPMI 1640 supplemented

with 25 mM HEPES, 10% (v/v) heat-inactive fetal calf serum (FCS) (Biowhittaker, Wokingham, UK), 10 mM glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin (components from Sigma Chemical Co., St. Louis, MO). For mRNA analysis cells, total RNA was extracted and DNAse-treated after TriPure isolation reagent (Roche Diagnostics GmbH, Mannheim, Germany) following manufacturer's instructions. Murine ADNP primers were derived from the published sequence of murine ADNP and reverse transciption polymerase chain reaction (RT-PCR) experimental conditions were previously reported.²³ cDNA was previously titrated to amplify in the linear range. Cytokine levels were determined by enzyme-linked immunosorbent assay (ELISA) according to manufacture's instructions (BD-Pharmingen, San Diego, CA).

RESULTS AND DISCUSSION

RT-PCR of mRNA using the ADNP primers from RAW 264.7 macrophages resulted in single DNA band when analyzed by agarose gel electrophoresis (Fig. 1 A). RT-PCR reactions were also processed with control β-actin house-keeping gene primers. These RT-PCR reactions correspond to the predicted size for PCR amplification using the ADNP primers, and nucleotide sequences of the amplified fragments showed an identical sequence to the mouse ADNP gene.⁵ Thus, we report for the first time that ADNP mRNA is expressed in immune system cells, namely, macrophages in a resting state. To further investigate the physiological role of ADNP in this context, we tested whether VIP treatment might influence ADNP gene expression in macrophages. Figure 1 A shows that ADNP mRNA levels were increased after 24 h of VIP treatment. The highest increase in ADNP mRNA was produced at nanomolar concentrations of VIP, with a slightly increased level at 10⁻¹² M VIP. The effect of VIP was not dose dependent, most probably due to VIP receptor desensitization in macrophages.²⁴ Thus, ADNP is a VIP-responsive gene in macrophages at concentrations that can be sensed by VIP receptors (VPAC) on immunocompetent cells. VIP production and secretion are elevated after immunological stimuli²⁵ and, therefore, some of VIP's immunomodulatory properties might be mediated in part by ADNP. To know whether ADNP mRNA levels are modified by immunological stimuli, we incubated RAW 264.7 cells in the presence of increasing concentrations of lipopolysaccharide (LPS) (0.1-10 mg/mL) for 24 h. ADNP gene expression was not altered after LPS treatment (Fig. 1 B). Toll-like receptors (TLRs) are key regulators of innate immunity, sensing and responding to invading microorganisms. LPS is the main ligand of the TLR-4 and TLRs include up to 10 different gene products.

So, at this point, we cannot rule out whether ADNP is regulated by other TLR-ligands. Nevertheless, these data should be taken as qualitative, taking into consideration the limitations of the RT-PCR approach to quantify

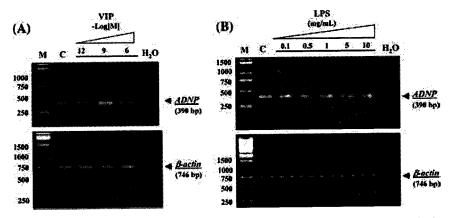


FIGURE 1. ADNP is expressed in macrophages. (A) RT-PCR analysis of ADNP in the macrophage-like RAW 264.7 cell line. Arrows indicate the PCR product amplified with specific ADNP primers (390 bp [base pairs]) and β-actin primers (746 bp). (B) Effect of LPS treatment on ADNP mRNA levels in RAW 264.7 cells. Results are representative of four independent experiments.

accurately mRNA. In this sense, differential expression of ADNP mRNA is currently being studied in our lab to assess VIP and known TLR-ligands effects by real-time PCR.

Given the breath of NAP's neuroprotective activities and the fact that ADNP is a VIP-responsive gene expressed in macrophages, we were interested to examine direct consequences of NAP treatment on key cytokines involved in the inflammatory response such as TNF-α, interleukin-6 (IL-6), and IL-12. RAW 264.7 cells treated with 0.1 mg/mL LPS in the presence of increasing concentrations of NAP for 24 h showed an inhibition of TNF-α, IL-6, and IL-12 secretion (Fig. 2). Although the mechanism of action involved is not yet known, our results support the role of NAP as a potent immunomodulator. Several questions related to the mechanism of action are under current investigation; we wish to learn whether ADNP could act in a paracrine and/or autocrine fashion under different circumstances. A study on NAP potential new functions can define novel mechanisms that modulate immune responses, and might lead to the development of new therapies for immune-mediated disorders, particularly, for neurodegenerative diseases in which neuronal defense mechanisms and immunomodulation represent innovative approaches.

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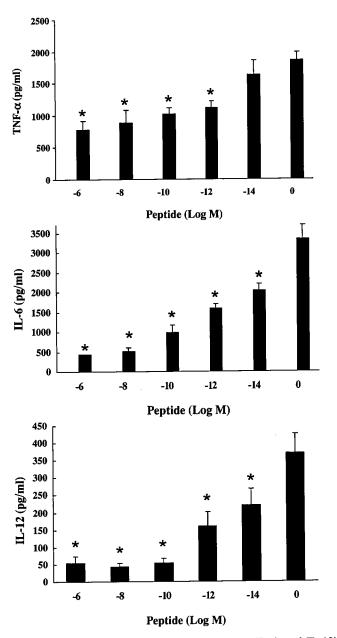


FIGURE 2. Effect of NAP on cytokine release (TNF- α , IL-6, and IL-12) by RAW 264.7 cells. Cytokine levels were determined in supernatants by ELISA after activation of RAW cells with 0.1 mg/mL LPS for 24 h in the presence of different concentrations of NAP. Cytokine basal levels for TNF- α , IL-6, and IL-12 were 485 \pm 83 pg/mL, 337 \pm 101 pg/mL, and 60 \pm 27 pg/mL, respectively. Statistical significance was determined by ANOVA followed by a Student-Newman-Keuls test.

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